PATENT COOPERATION TREATY

REC'D 28 FEB 2005
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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference NEO001PCT	FOR FURTHER ACTION See Form PCT/IPEA/416	
International application No. PCT/JP2004/004378	International filing date *(day/month/year)* 26.03.2004	Priority date *(day/month/year)* 28.03.2003
International Patent Classification (IPC) or national classification and IPC C12N15/10, C12Q1/68		
Applicant NEO-MORGAN LABORATORY INCORPORATED et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 12 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
22.10.2004	25.02.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Schmitt, C Telephone No. +49 89 2399-7351

Form PCT/IPEA/409 (Cover Sheet) (January 2004)

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-214 as originally filed

Sequence listings part of the description, Pages

1-236 as originally filed

Claims, Numbers

1-124 as originally filed

Drawings, Sheets

1/19-19/19 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-90, 95, 97, 99, 115, 116 and 123

because:

☒ the said international application, or the said claims Nos. 1-90, 95, 97, 99, 115, 116 and 123 relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	117-122
	No:	Claims	1-116, 123,124
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-124
Industrial applicability (IA)	Yes:	Claims	91-94, 96, 98, 100-114, 117-122,124
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re Item I

Basis of the report

The basis for this report is the application as originally filed.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Independent claims 1, 45, 90, 95, 97, 99, 115 and 116 relate to a method for regulating a conversion rate of a hereditary trait of a cell, a method for producing a cell having a regulated hereditary trait, a method for producing an organism having a regulated hereditary trait, a method for producing a nucleic acid molecule comprising changing an error-prone frequency of a gene replication of an organism, a method for producing a metabolite of an organism comprising changing an error-prone frequency of gene replication of an organism, a method for testing a drug comprising using a cell and a method for testing a drug comprising using an organism, respectively.
Said claims encompass in vivo methods since they are not limited to isolated cells. Moreover,taking into account the fact that the term "a cell" refers not only to a single cell but also to a plurality of cells (e.g. page 29, lines 21-26) and therefore encompasses whole organisms such as humans or animals, said claims encompass a step of treatment by surgery practised on the human/animal body.
Claims 1 ,45, 90, 97 and 99 relate therefore to subject-matter considered by this Authority to be covered by the provision of Rule 67.1 (iv) PCT.
The same applies to claims 2-44 and 46-89 which are dependent on claims 1 and 45, respectively.

Independent claim 123 relates to the use of at least two kinds of polymerases for regulating a conversion rate of an hereditary trait of an organism. Said claim is thus regarded as relating to a method of treatment by therapy practised on the human or animal body.
Claim 123 relates therefore to subject-matter considered by this Authority to be covered by the provision of Rule 67.1 (iv) PCT.

Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of claims 1-90, 95, 97, 99, 115, 116 and 123

(Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents are referred to in this communication:

D1 : MORRISON ALAN ET AL: "The 3'-to-5' exonucleases of both DNA polymerases delta and epsilon participate in correcting errors of DNA replication in Saccharomyces cerevisiae" MOLECULAR AND GENERAL GENETICS, vol. 242, no. 3, 1994, pages 289-296, XP001183125 ISSN: 0026-8925.

D2 : FURUSAWA MITSURU ET AL: "Asymmetrical DNA replication promotes evolution: Disparity theory of evolution" GENETICA (DORDRECHT), vol. 102-103, no. 0, 1998, pages 333-347, XP009036173 ISSN: 0016-6707.

D3 : IWAKI T ET AL: "PREFERENTIAL REPLICATION-DEPENDENT MUTAGENESIS IN THE LAGGING DNA STRAND IN ESCHERICHIA COLI" MOLECULAR AND GENERAL GENETICS, SPRINGER VERLAG, BERLIN,, DE, vol. 251, 1996, pages 657-664, XP002926094 ISSN: 0026-8925.

D4 : EP 1 054 057 A (JAPAN SCIENCE &; TECH CORP) 22 November 2000 (2000-11-22).

D5 : AOKI K ET AL: "Promotion of evolution by intracellular coexistence of mutator and normal DNA polymerases." JOURNAL OF THEORETICAL BIOLOGY. 21 MAR 2001, vol. 209, no. 2, 21 March 2001 (2001-03-21), pages 213-222, XP002295420 ISSN: 0022-5193.

D6: GOLDSBY ROBERT E ET AL: "High incidence of epithelial cancers in mice deficient for DNA polymerase delta proofreading." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA. 26 NOV 2002, vol. 99, no. 24, 26 November 2002 (2002-11-26), pages 15560-15565, XP002296102 ISSN: 0027-8424.

D7: MORRISON A ET AL: "PATHWAY CORRECTING DNA REPLICATION ERRORS

IN SACCHAROMYCES CEREVISIAE" EMBO JOURNAL, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 12, no. 4, 1993, pages 1467-1473, XP001145623 ISSN: 0261-4189.

V.1.

a. Document D4 (the references in parentheses applying to this document) discloses a method for randomly mutagenizing a gene comprising introducing much more mutations into one strand of double-stranded genomic DNA of a cell or a organism than into the other strand (paragraphs [0009]-[0010]). Said method is therefore considered as a method for regulating a conversion rate of a hereditary trait of a cell or a method for producing a cell having a regulated hereditary trait. The cell or organism is a mutant cell strain or a mutant organism having a mutator gene in a mutation repair mechanism gene group (paragraph [0011]). The mutator gene is preferably dnaQ, i.e. ε subunit of DNA polymerase III, or dnaE, i.e. α subunit of DNA polymerase III (paragraph [0012]). The mutant cell or organism may inherently have the mutator gene or the mutator gene can be introduced in said cell or organism (paragraph [0011]). *E.coli* dnaQ49 strains are examples of such mutant cell (paragraph [0033]). More preferably, the gene which causes a defect in mutation repair is a thermosensible mutator gene (paragaphs [0014]-[0015]).
In a preferred embodiment, mutations are introduced into genomic DNA under certain conditions where no selected pressure is applied and mutants are then selected under pressure condition (paragraphs [0015] and [0031]). For example, mutants resistant to ampicillin antibiotic can be selected (paragraph [0033]).
The mutation rate to be induced is preferably within a range of 100- to 100,000-fold of natural mutation (paragraph [0029]).
Lastly, mutated gene which is isolated from such a mutant cell or organism is also claimed (claim 10).

b. The applicant's attention is brought to the fact that the use of the expression "corresponding DNA polymerases thereto" in claims 13-15, 18, 58-60 and 63, and the use of the term "variant" in claims 103 and 104 implies that any DNA polymerase falls under the scope of said claims. In addition, it is to be noted that any prokaryotic or eukaryotic cell possesses at least two different DNA polymerases having different proofreading activities (i.e. for example in *E.coli*, DNA pol I possesses 3'-to-5' exonuclease activity and polymerase activity whereas the α-subunit of DNA pol III only possesses polymerase activity). Furthermore, it is

to be noted that the method for mutagenesis followed by a selection of mutants which are resistant to ampicillin can be considered as a method for testing a drug. Lastly, it is to be noted that product-by-process claims 96, 98, 100 and 112 which are directed to a nucleic acid molecule, a polypeptide, a metabolite and a product substance, respectively, are so broadly defined that they encompass any nucleic acid molecule, polypeptide, metabolite and product substance, respectively.

c. Thus, all the features of claims 1-27, 30, 33-38, 41-72, 75, 78-97, 99-101, 103, 104, 106, 110-116, 123 and 124 are anticipated by document D4.
Said claims are therefore not new in the sense of Article 33(2) PCT.

V.2.

a. Document D1 (the reference in parentheses applying to this document) discloses a method for increasing the rate of mutations in yeast cells by using DNA polymerase δ and ε 3'-to-5' exonuclease-deficient mutants denoted *pol3-01* and *pol2-4*, respectively (abstract). These DNA polymerase δ and ε 3'-to-5' exonuclease-deficient mutants do not have cell growth defect (page 289, col.2, last paragraph). Plasmids containing the pol3-01 or pol2-4 fragments were constructed (page 290, col. 1, sections "plasmids" and "construction of yeast strains") and were introduced into the yeast chromosome by targeted integration (see document D7 on page 1472, col.1 for further details. Said document has been cited in document D1 and its disclosure is considered as being incorporated into document D1). *pol3-01* and *pol2-4* increase mutation rate by factors of the order of 10^2 and 10^1, respectively (abstract).
Mutation rates are measured as *URA3* forward mutation (i.e. FOA resistant mutant) and *his7-2* reversion (abstract). FOA^r mutant yeast cells are amplified by PCR and sequenced (page 290, col.2, last paragraph), implying that the DNA molecule containing the mutation is isolated.
Lastly, 3'-to-5' exonuclease activity of DNA polymerases δ and ε act on opposite strands, which implies that the proofreading activity during replication is different between the leading and the lagging strands (abstract).

b. Taking into account the above item V.1.b., all the features of claims 1-31, 33-38, 42-76, 78-108, 110-116, 123 and 124 are considered to be anticipated by document D1. Claims 1-31, 33-38, 42-76, 78-97, 99-108, 110-116, 123 and 124 are therefore not new in the sense of Article 33(2) PCT.

V.3. Furthermore, taking into account the above item V.1.b., claims 1-97, 99-108, 110-114, 123 and 124 are also considered to be anticipated by the disclosure of document D6, which discloses a method for increasing the spontaneous mutation rate of mouse cells by inactivating the 3'-to-5' exonuclease activity of DNA polδ (see relevant passages cited in the International Search Report). Said document discloses, in addition, that a baculovirus system is used to express and purify recombinant wt and D400A polδ p125 proteins (page 15561, col.2, section "polymerase and exonuclease assays"). Said D400A polδ p125 protein is considered as falling under the scope of claims 98, 100 and 112.

Claims 1-108, 110-114, 123 and 124 are therefore not new in the sense of Article 33(2) PCT.

V.4. Document D8 (the references in parentheses applying to this document) discloses Exo-deficient polIII mutants of *Bacillus subtilis* which have mutator phenotype (abstract and page 45, col.2, last paragraph-page 46, col.1, first paragraph). A mutated version of *polC* designated *mut*-1A and which affects the exonuclease function of PolIII is introduced into *B. subtilis* by transformation. Transformant carrying the *mut*-1A mutation are compared with equivalent isogenic wt transformants for the presence of a mutator phenotype by determining the incidence of colonies resistant to two different antibiotics (page 46, col.2, 1st paragraph and Table I). *mut*-1A transformants display an incidence of resistant colony development 10^3-10^4 times that of the wt.
Furthermore, two mutant forms of *B. subtilis* PolIII denoted Exo1 and Exo2, respectively, are overexpressed in *E.coli* to assess the catalytic activities of the products thus obtained (section (1) selective suppression of *BsPolIII* Exo Activity of Results and discussion). Said proteins are considered to fall under the scope of claims 98, 100 and 112.

All the features of claims 1-28, 30, 33-38, 41-73, 75, 78-83, 86-114, 123 and 124 are anticipated by document D8. Claims are therefore not considered new in the sense of Article 33(2) PCT.

V.5. Claims 117-122 are considered to be new in the sense of Article 33(2) PCT as their features are not disclosed in any available prior art. However, said claims are not considered inventive in the sense of Article 33(3) PCT.
Said claims relate to a set of at least two kinds of polymerases **suitable** for use in

regulating a conversion rate of a hereditary trait of an organism or in producing an organism having a regulated hereditary trait. Therefore, the packaging of known polymerases into a set would be obvious to the skilled person.

Re Item VIII

Certain observations on the international application

The application does not meet the requirements of Article 6 PCT, because claims 1-124 are not clear.
The reasons being that either the claims attempt to define the subject-matter in terms of the result to be achieved, which merely amounts to a statement of the underlying problem, without providing the technical features necessary for achieving this result and/or the claims attempt to define the subject-matter in terms of functional features, which do not enable the skilled person to determine which technical features are necessary to perform the stated functions.

Concluding remarks.

Should the applicant proceed with an International preliminary examination of the present application, his attention is brought to the fact that a negative IPER will be issued on the basis of the present claims.

The applicant's attention is drawn to the fact that, as a consequence of Rule 66.8(a) PCT, the examiner is not permitted to carry out any amendments under the PCT procedure, however minor these may be.

Independent claims 1, 45, 123 and 124 are considered to lack novelty under Article 33(2) PCT (see above). Should the applicant overcome these objections for lack of novelty, then the question of inventive step of said claims should be discussed, especially with respect to the disclosure of documents D2 or D5. When filing a reply, the applicant should therefore take into consideration the disclosure of document D2 which discloses that evolution of living things, including multicellular organisms, may be accelerated by artificially increasing the fidelity difference between the leading and lagging strand using a disparity mutator (abstract, page 346, col.2) and the disclosure of document D5 which discloses a simulation with a genetic algorithm showing that bacteria replicating with a

disparity strategy (coexistence of a mutator polymerase with low fidelity and a normal polymerase with high fidelity) undergo rapid evolution (abstract and discussion).

Further remarks.

Should the applicant enter the regional phase, his attention is brought to the fact that independent claims 1, 45, 90, 91, 93, 95, 97, 99, 106 and 111 may not fulfill the requirements for patentability in certain regional offices. For example, the EPO considers that Independent product claims 91, 93, 106 and 111 encompass human beings and thus are not patentable under Article 53(a) EPC.

Similarly, independent claims 1, 45, 90, 95, 97 and 99 which relate to a method for regulating a conversion rate of a hereditary trait of a cell, a method for producing a cell having a regulated hereditary trait, a method for producing an organism having a regulated hereditary trait, a method for producing a nucleic acid molecule comprising changing an error-prone frequency of a gene replication of an organism and a method for producing a metabolite of an organism comprising changing an error-prone frequency of gene replication of an organism respectively, encompass either a step of modifying the germ line genetic identity of human beings or are directed to a process for modifying the germ line genetic identity of human beings and are considered to be not patentable under Article 53(a) and Rule 23d EPC.